

The healthcare sector is alarmed by the issues of antimicrobial resistance/drug resistance. There are very few FDA approved anti-MRSA antimicrobial agents that we can rely upon. Researchers all over the world are engaged with metabolic engineering and genetic improvement of these life-saving drugs that can combat drug resistance. But, there is an urge to improve their production to reduce the cost of their industrial applicability. Therefore, benefiting a larger section of the society. Daptomycin is one such novel cyclic lipopeptide antibiotic produced by *Streptomyces roseosporus*, which works effectively against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococci* due to its unique mechanism of action. This antibiotic received its FDA approval in 2003 for the treatment of patients with structure skin infections, right-sided endocarditis, and bacteremia. The filamentous nature of *S. roseosporus* affects the rheology and hence hampers its production significantly. Researchers have attempted to increase the yield through genetic engineering and metabolic flux analysis. Very few attempts have been there towards the cell processing strategies for improved Daptomycin production.

The physiological state and morphological differentiation of filamentous microorganisms can be related to changes in growth conditions during submerged cultivations. Morphological features have been correlated with the production of antibiotic at several instances. Improved antimicrobials' production was observed through pellet formation. Also, whole-cell immobilization is a useful processing strategy to instantiate the potential of bioprocesses. Previous reports have contributed to explore how the phenomenon of immobilization affects growth and metabolite production. Higher cell concentration can be maintained by

immobilization methods which enhance the productivity of the antibiotic as it is mainly a non-growth associated product. Prolonged reusability is also an advantage of whole-cell immobilization. Therefore, the production of Daptomycin was evaluated through improved broth rheology and controlled hydrodynamics. Therefore, some alternative strategies were attempted to improve Daptomycin production.

The fermentation media was optimized using the One factor at a time (OFAT) method. The optimum temperature and pH were found to be 30°C and 7.0, while the appropriate inoculum size was 5% (v/v) and most favorable age of inoculum was 48 h. Dextrin served as best carbon sources for Daptomycin production, whereas Soybean meal was an effective nitrogen source which resulted in increased Daptomycin production. After the manual optimization, Statistical optimization by Taguchi method was done to screen the optimal concentration of the media components for the enhanced Daptomycin production. Final medium for Daptomycin production is (g/l), Dextrin 30, Glucose 10, Soybean flour 20, Fe (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub> 0.6 and KH<sub>2</sub>PO<sub>4</sub> 0.2 and pH 7; incubated at 30 °C for 6 days. To verify these results, experiments were carried out at the given concentrations of media components and Daptomycin production was 315mg/L as compared to 320 mg/L predicted using MINITAB 17. This showed that the experimental value almost matches with predicted values. The optimized medium gave 315 mg/L of Daptomycin as compared to 200 mg/L prior optimization.

The morphology of the producer i.e. *S.roseosporus* was evaluated microscopically and it was seen that during the stationary phase, the morphology of *S. roseosporus* was substantially changed in submerged culture due to the rapid secretion of Daptomycin. Among several Newtonian and Non-Newtonian models of rheological models, the rheological data for

Daptomycin fermentation were best fitted in the Power Law Model. At low shear stress condition, the consistency index (K) increased up to 0.28 Pa<sup>-sn</sup> on the fifth day and then decreased to 0.13 Pa<sup>-sn</sup> on the sixth day. This condition was contradicted by the results seen at higher stress condition wherein rapid changes in K and n were revealed. The flow behavior index or the power-law index (n) decreased with increasing stress with the passage of time indicating the typical shear thinning of culture broth due to complex morphological alterations of actinomycetes. Morphological alterations lead to the viscosity of the broth, increased respiration rate and need for proper oxygen mass transfer leading to stress conditions. This stress condition eventually affects the bioprocessor fermentative production of the antibiotic in a negative manner. Therefore, the study of the rheological patterns of the microorganism using different mathematical models helped to track the nature of the fermentative broth and hence to take up measures to increase mass transfer rate through various processing strategies. Daptomycin production in stirred tank bioreactor was not found advantageous as *Streptomyces roseosporus* were shear sensitive and the broth hydrodynamics was affected due to viscous nature of broth.

Whole cell immobilization is an important phenomenon to deal with the problem of viscosity and shear sensitivity of cells. Therefore, immobilization studies were attempted in a packed bed bioreactor using *Streptomyces roseosporus* immobilized in calcium alginate beads, silk sachets, and loofah sponge. The structural analysis of the porous matrices was done using Scanning Electron Microscopy (SEM). 1.5 grams of cells per grams of the carrier was found to be suitable for whole cell immobilization studies in an indigenously designed and developed packed bed bioreactor. Continuous production of Daptomycin was done in the bioreactor at fixed substrate concentration and at different dilution rates. The dilution rate

was varied from  $0.01 \text{ h}^{-1}$  to  $0.040 \text{ h}^{-1}$  substrate. The substrate consumption increased while the product formation depleted with increasing dilution rate. The highest productivity was accounted for the loofah sponge at a dilution rate of  $0.025 \text{ h}^{-1}$ . With varying dilution rates, the applicability of the whole cell immobilization improved substrate utilization and minimized the amount of substrate lost in the product stream. The residence time was increased due to cell immobilization which led to enhanced productivity and better substrate utilization.

Further, morphological variations of *S. roseosporus* cells were carried out for enhanced production of Daptomycin in an internal-loop airlift bioreactor. Free cells, pelletized cells and immobilized cells on ultra porous refractory brick and silk sachets were investigated for the production of Daptomycin in a lab-scale 2.0 l air-lift bioreactor. The effect(s) of nitrogen source, inoculum size, and oxygen stress were analyzed for pellet formation of *S. roseosporus*. Interestingly, free cells produced 750 mg/l of Daptomycin in a single batch. But, the three-phase broth viscosity increased due to vigorous growth of free cells which hampered the oxygen transfer rate. The cell degeneration over the time deterred pellet reusability. 1430 mg/l Daptomycin was produced using pellets for 2 batches. On the contrary, mechanical stability, buoyancy, and reusability of refractory bricks and silk sachets were beneficial. Daptomycin production was recorded for 6–8 batches. Immobilized cells on refractory bricks and silk sachets led to 4895 mg/l and 3623 mg/l Daptomycin production respectively. Cell immobilization improved the three-phase broth rheology and hence, the hydrodynamics within the reactor. Therefore, whole-cell immobilization could contribute to the ameliorated production of this life-saving drug.

The effects of the sparger designs on mass transfer characteristics in a lab-scale internal loop airlift bioreactor were evaluated to tackle mass transfer issues inside the internal-loop airlift bioreactor during the fermentation process. It was observed that gas holdup and volumetric oxygen mass transfer coefficient depends on sparger design and especially the superficial gas velocity. Comparative studies of the experimental data of the single hole sparger and the multiple hole sparger indicated that the sparger with the larger number of holes was more effective for the mass transfer as it generated a smaller mean bubble diameter and a larger number of bubbles leading to greater gas hold up values as compared to single hole sparger. Furthermore, for a specific sparger structure, the influence of the superficial gas velocity on the  $K_L a$  could be found on the specific interfacial area as interpreted using gas hold up values. Empirical relationships could estimate the hydrodynamic factors for the gas holdup values and the volumetric oxygen transfer coefficient for two different spargers. The empirical correlations were in sync with the experimental results found. Hence, it could be summarized that the bubble characteristics could be utilized to study hydrodynamics inside an airlift bioreactor and sparger geometry could be used to improve the oxygen mass transfer characteristics of the fermentation broth and hence to improve the production of Daptomycin.

As a part of the application of Daptomycin as an anti-MRSA antibiotic, the antimicrobial activities of the Daptomycin along with biosynthesized noble metal nanoparticles was investigated against methicillin-resistant *S. aureus*. Thus, promising its potentiality for therapeutic purposes, especially; skin infection treatments in future endeavors. Topical antibiotic ointments have been mainly used in the prevention of burn infections in comparison to other painful, surgical and therapeutic indications which could be prone to

infection. Such antimicrobials help in prevent further microbial proliferation and cure burn/wound infections. Another consideration is important that is the specificity towards pathogen and also toxicity towards the host cells. Gel-based formulations are favorable in general because they are greaseless, easily spreadable, effective and soothing. The process was non-toxic and eco-friendly. The synergistic effect led to the improved antimicrobial efficacy. Daptomycin along with gold nanoparticles, silver nanoparticles and bimetallic nanoparticles produced by *Aspergillus sp.* showed improved bactericidal activity than free form of antibiotic. There was 37% increase in efficacy of Daptomycin due to the synergistic oligodynamic effect with metallic nanoparticles. The synergistic form of the drug prompted that the toxicity of high drug dosage could be mitigated in an economical way through extensive studies in future.

Therefore, the present studies are a comprehensive analysis of the cell processing strategies for the improved production of Daptomycin using different immobilized modes in an airlift bioreactor and packed bed bioreactor. The mass transfer issues in the airlift bioreactor were addressed through bubble size optimization using different spargers and the combinatorial antimicrobial effect of Daptomycin with nanoparticles were also explored in this work. The future prospects would address the remaining technical and economical aspects of Daptomycin fermentation process and the topical use of this crucial drug. However, the strategic production of Daptomycin using varied cell processing methods would be beneficial and likely to be used in future with a significant advantage. The downstream processing issues would be taken care as a part of future studies on Daptomycin production, which is definitely a crucial concern. Also, in future, much more efforts are to be taken to establish the

combinatorial therapy of Daptomycin with metal nanoparticles as an anti-MRSA agent which was briefly dealt in the present work.